The Origin and Structure of Mast Cells in the Adult Rabbit.

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The mast cell is a common and conspicuous cell element of all mammals, occurring in varying relative proportions in the loose connective tissues and the blood. Waldeyer first undertook the investigation of the cells of the loose tissues and designated the larger cells with abundant protoplasm as plasma cells. The interstitial cells of the testis, cells of the carotid and coccygeal glands, advential cells of the vessels of the brain, cells of the suprarenal, corpus luteum and the decidual cells of the placenta were all included among the plasma cells. Later, Unna detected a type of cell which differed from all the others described by Waldeyer and restricted the term plasma cell to this type. Ehrlich, in his investigation of the cells of the loose connective tissues, observed that certain of Waldeyer's cells contained granules which had remarkable affinity for basic anilin dyes. In this respect, they were, therefore, very different from the non-granular, strongly basophilic cells

which have usually been included among the plasma cells. Ehrlich found that these granular cells were very numerous in the neighborhood of the blood vessels where the conditions of nutrition were favorable. For this reason, he believed that the over-nourishment (Mästung) of certain of the connective tissue cells in this region was the cause of their progressive differentiation into the basophilic granular cells. They were called "Mastzellen" on account of their supposed relation to the conditions of nutrition. Later, Ehrlich and Westphal showed that cells containing basophilic granules were present in the blood also. On account of their staining reactions, Ehrlich and Westphal included them among the mast cells. They regarded the mast cells of the blood as having their origin in the bone marrow, but otherwise they failed to distinguish between the blood mast cells and the mast cells of the connective tissues. According to Gulland and Türk, the only difference between the mast cells of the blood and those of the connective tissues is merely one of size. However, Maximow, Weidenreich and Pappenheim have

shown that in mammals the fully differentiated histogenous mast cell is absolutely distinct from the fully differentiated mast cell of the blood. The histogenous mast cells are confined to the connective tissues, however, in the rat and mouse they may occur in the peritoneal transudation, but never in the circulating blood. The haematogenous mast cells, on the contrary, are not limited to the blood, but occur in great numbers in the connective tissues, which is readily seen in the rabbit. Maximow and Weidenreich have demonstrated that the only characteristic which the two types of cells have in common is the presence of basophilic granules in the cytoplasm, which stain metachromatically with basic annilin dyes. Maximow finds that with the same fixation and stain the basophilic granules in the two types are not equally well preserved, the granules of the haematogenous cells being the more soluble in water. Maximow and Weidenreich have shown further that the nuclei of the two types do not present the same configuration; in the histogenous cell the nucleus is always round or oval, while, in the

haematogenous type of cell it may be divided into several distinct lobes, or it may be kidney shaped. The only feature in common is the presence of basophilic granules, which have a strong affinity for basic dyes, but aside from this the two types of cells represent independent lines of leucocyte differentiation and development, although they may be associated in their functions. The same authors have shown that even in the same animal there may be variation between the histogenous and haematogenous cells in the size and shape of the granules. Weidenreich found that the haematogenous mast cells of different animals vary in size, structure, and staining reactions. He distinguishes a guinea pig type of blood mast cell and a human type. In the guinea pig type he regards the granulation as a real specific granulation, while the granules in the human type are formed from particles of the fragmenting nucleus.

Maximow emphasizes the fact that where there are comparatively few haematogenous mast cells the deficiency is made up for by the increased numbers of histogenous mast cells and vice versa. The mouse may be mentioned as

having a great many connective tissue mast cells, with a corresponding diminution in the number of blood mast cells. In the rabbit conditions are just the reverse, there being many of the former and few of the latter.

In the lower forms it is generally conceded that the two types of mast cells are closely related and even identical. Maximow reports that in the axolotl, blood mast cells have actually been seen migrating out from the blood vessels and were being transformed into connective tissue mast cells. Maximow is of the opinion that in the non-mammalia the two types are identical. According to the researches of Dantschakoff the two types of mast cell are identical in reptilia and birds, both in the embryo and in the adult.

The old assumption that the two types of mast cells were identical is no longer valid. The investigations of Heidenreich and Maximow show conclusively that the histogenous type of mast cell is distinct from the haematogenous, that they are distinct types of cells, with their own peculiar nuclei , granules and staining reactions.

In regard to the origin of the haematogenous mast cell the following questions have arisen: (1) is the mast leucocyte to be placed on the same level with the other granular cells? (2) Is the granulation a real specific granulation?

If the mast leucocyte is a true granular cell we should expect to find the basophilic myelocyte stage in the bone marrow and also the fully differentiated cell. Ehrlich and Shridde derive mast leucocytes from basophilic myelocytes in the bone marrow and state that they have the same evolution as the other granular cells. Jolly has described mast leucocytes in the bone marrow of the guinea pig and has seen them in mitosis. In the bone marrow of the cat, however, he was unable to find mast leucocytes. According to Blumenthal mast leucocytes originate in the bone marrow.

Pappenheim opposes Ehrlich's theory, namely that mast leucocytes originate in the bone marrow. In the first place, he does not believe that the mast granulation is as specific as is that of the eosinophil and

pseudo-eosinophil as Ehrlich holds, but that the granules are derived from the degenerating spongioplasm of a lymphocyte within the circulation with an addition of chromidial substance. Pappenheim holds that the mast leucocyte is not represented in the bone marrow of man and rabbit, but that the mast leucocyte is a degenerate type and cannot be placed on the same level with the other granular cells.

Weidenreich found that the nucleus of the human mast leucocyte is very polymorphous in myelogenous leukemia where there is a general leucocytosis with many myelocytes, one would expect to find mast myelocytes also. However, Weidenreich found that instead of more mast leucocytes with a round nucleus there were more with a polymorphous nucleus. The fact that the nucleus becomes more polymorphous than in normal blood, proves that the human mast leucocyte does not have a myelocyte stage. Weidenreich concludes that in man haematogenous mast cells are formed from degenerating lymphocytes, the granules being formed by the breaking up of the nucleus. He further found that in man the mast leucocyte does not represent a true granular cell, but "für den Typus Meerschweinchen (guinea pig)

dagegen nicht, und wie sich andre Säuger hierin verhalten, bedarf noch der Prüfung."

Prüscher claims that the mast leucocytes of the rabbit are formed from altered lymphocytes within the circulation. The author obtained a mast leucocytosis by injecting a blood poison into the circulation and concluded that mast leucocytes are formed in the circulation from lymphocytes by a mucoid degeneration of the spongioplasm. He concludes that the formation of the mast granules is associated with the mucoid degeneration of the spongioplasm of the lymphocyte, and that the nucleus is not concerned in the elaboration of the basophilic mast granules as it presents no signs of fragmentation, neither were nuclear buds being extruded into the cytoplasm. The author concludes that "Auf Grund unserer experimentellen Beobachtungen müssen wir also Annehmen dass die basophile Leukozytose nicht durch Auswanderung der im Knochenmarch sessilen basophile Myelozyten, sondern durch einen (toxisch) degenerativen Prozess der hämatischen Lymphocyten zustande kommt."

According to Kardos, the bone marrow both of the guinea pig and the rabbit, contains neither mast myelocytes nor fully differentiated mast leucocytes, therefore, he thinks it possible "dasz die Kaninchenmastzellen im Blut selbst aus Lymphozytenvorstufen enstehen. Die sog. Blutmastleukozyten stammen natürlich aus dem Knochenmark, aber z.T. sind sie keine eigentlichen Mastzellen, sondern nur unreifkörnige sonstige granulozyten, deren granula eine andre chromophile Reaktion hat, z.T. soweit sie eigentliche Blutmastzellen sind, bilden sie sich aus Lymphoidzellen wohl erst im Blue selbst oder unter pathologischen Einwirkung."

Banacchio finds basophilic myelocytes in the bone marrow of the rabbit with a varying number of granules which at a casual glance resemble what we should expect to find as the precursor of the fully differentiated mast leucocyte. He decides that the basophilic myelocytes which are present are merely unripe eosinophiles and pseudo-eosinophiles, with a primitive granulation which in its early stage is predominately basophilic. During the process of

differentiation the basophilic granulation is gradually transformed into the permanent eosinophilic granulation. Benacchio describes and illustrates the process of differentiation from the early basophilic myelocyte to the final eosinophil and pseudo-eosinophil. The author did not determine the origin of the mast leucocytes in the rabbit and guinea pig, but he is positive that they do not arise in the bone marrow. The basophilic myelocytes which other authors have described as mast myelocytes are in reality only pseudo-eosinophilic and eosinophilic myelocytes, in which the recently formed granules are basophilic.

Pappenheim and St.Szecsi obtained a leucocytosis of the special cells by injecting saponin into the circulating blood of the rabbit. However, the injection did not cause an increase in the number of mast leucocytes. The circulating blood of these rabbits contained basophilic myelocytes and all the intermediate stages which Benacchio described in the bone marrow as unripe eosinophiles and pseudo-eosinophiles. They conclude, in agreement with Benacchio, "Blutmastzellen sind also keine eigentliche zellart, sondern entweder abortivkörnige, sonstige Granulozyten."

In direct opposition to the theories of Benacchio and Pappenheim, Maximow claims that the haematogenous mast cell of the rabbit is a true granular cell equivalent to the other granular cells, and is not derived from a lymphocyte in which the spongioplasm has undergone a mucoid degeneration and thus given rise to the mast granules as Pappenheim holds for the mast leucocyte of the rabbit. Maximow believes, and as already stated, that the mast leucocyte of the rabbit has its own peculiar and characteristic evolution in the bone marrow from a cell with a small round nucleus with a varying number of basophilic granules in the cytoplasm. He states that in embryo rabbits of 43-46 mm. mast myelocytes originate in the bone marrow and connective tissues and at the same time from lymphocytoid wandering cells.

The above brief resumé of the literature applies only to the origin of the mast leucocyte. In order to avoid confusion the histogenous and haematogenous mast cells will be considered separately in the following report of my investigation.

At this point I wish to express my indebtedness to Professor Hal Downey for his many helpful suggestions and for the inspiration afforded by the constant enthusiastic interest shown by him during the progress of this investigation.

In this investigation the ordinary domestic rabbit was used. Owing to the solubility of mast cell granules Maximow recommends alcoholic fixation and this is the method that was followed. Portions of fresh bone marrow were fixed in 100% alcohol from four to five hours and embedded in paraffin. Sections were cut 4 micra thick, placed on cover glasses and stained in a saturated solution of thionin in 50% alcohol for forty-eight hours; other sections were stained in May Giemsa. Bone marrow was also fixed in the following:- Zenker's fluid to which 10% formol was added; a saturated solution of $HgCl_2$ in a 0.9% solution of NaCl; a combination of $HgCl_2$, 0.9% NaCl, and 10% formol. Preparations fixed in 100% alcohol, followed by staining in a saturated solution of thionin in 50% alcohol proved to be most favorable for study, as

the basophilic granulation is well preserved. Bone marrow smears were prepared according to Pappenheim's method. A small drop of a normal salt solution was placed in the center of a cover glass and with the fine forceps a small piece of bone marrow was brought into contact with the solution and drawn out into a thin film by a second cover glass. Preparations were stained in May Grünwald for three minutes, five drops of Giemsa in 10 cc. of distilled water was then added and the mixture allowed to stand for eight minutes. Smears were then dried between filter paper and mounted in damar. The fixation in this method was obtained by means of the methyl alcohol of the May Grünwald solution.

Blood smears were made by pricking the ear of the rabbit and spreading the small blood drop into a thin film. Some smears were fixed, while still moist, in 100% alcohol from three to fifteen minutes and stained in a saturated solution of thionin in 50% alcohol. Others were fixed over osmic fumes, and still others in the fumes of formalin and stained with either Wright's or May Giemsa.

For the study of the histogenous mast cell, portions of the abdominal wall of the rabbit were pinned out

flat on small pieces of cork, some were fixed in 100% alcohol, others in Zenker-formol. Preparations of the abdominal wall were slowly embedded in celloidin. Maximow recommends cutting sections parallel with the surface as the cells are large and appear in their greatest length when so sectioned, and this was done. The sections were stained in 50% and 80% alcoholic thionin respectively. Methyl Green Pyronin and Iron Haematoxylin were also used.

Mast leucocytes are unusually numerous in the rabbit. According to Brinckerhoff 4 to 8% of the total number of leucocytes in the circulating blood of the rabbit are mast leucocytes. Numerous mast leucocytes are also present in the loose connective tissues.

Figures 1 and 2 from alcoholic thionin preparations illustrate typical mast leucocytes of the rabbit. The basophilic granules which are characteristic of these cells vary in form, size, number, and frequently in the staining intensity. In the rabbit the granules are very fine, usually rounded or slightly irregular, and very numerous, often making it difficult to see the exact outline

of the nucleus. Maximow points out the fact that the granules in the haematogenous mast cell of the rabbit are extremely soluble in water, and therefore he recommends alcoholic fixation and alcoholic stains.

The configuration of the nucleus shows great variation. According to Maximow, "Der Kern hat meist die Form eines zusammen geknickten Schlauches von unregelmässige Dicke mit abgerundeten, oft keulenförmig aufgetriebenen Enden, und oft sehr tiefen Einschnurungen Figure 5 from a smear, fixed in formol vapor and stained in Giemsa shows the exact boundaries of the nucleus, the granules having been dissolved. Pröscher claims that the nucleus of the rabbit mast leucocyte remains practically identical with the lymphocyte nucleus from which it is derived. In the majority of mast leucocytes the nucleus is covered by basophilic granules, but in cases where the exact outlines could be seen, I found that it is typically polymorphous and showed no similarity to the lymphocyte nucleus, neither did it possess lymphocyte characters nor signs of degeneration. Pröscher is of the opinion that in

the rabbit the nucleus is not concerned in the elaboration of the mast granules, as is claimed by Weidenreich for the mast leucocyte of man. In my investigations I have seen nothing that would indicate that the nucleus participated in the formation of mast granules.

The bone marrow being the source of granular cells, it was natural to turn to it for the study of the parent cell of the mast leucocyte. In the meshes of the reticular tissue of the bone marrow there are various kind of cells. The great majority are the myelocytes, which evolve into granular cells. In addition to the myelocytes there are megakaryocytes , erythrocytes and lymphocytes. According to the investigations of Wright and Downey, the megakaryocytes are found to give origin to the blood platelets. Erythrocytes, in various stages of differentiation, are numerous and usually appear in smaller or larger clusters, as the case may be; lymphocytes are not common.

The myelocytes, or the precursors of the granular cells, including neutrophiles and eosinophiles,are of particular importance in this investigation. If the mast

leucocyte of the rabbit is a true granular cell we should expect to find it represented in the bone marrow by a myelocyte with basophilic granules, and also, by at least a few of the fully differentiated cells. Myelocytes are confined to the bone marrow, but in certain pathological cases, such as myelogenous leukemia, they enter the circulating blood in great numbers. Normally they do not occur outside of the bone marrow. The myelocyte or marrow cell takes a conspicuous place in the histological picture of the bone marrow, since it is concerned with the production and differentiation of all the granular cells. The typical myelocyte possesses a round nucleus in the early stage, with a variable number of granules distributed throughout the protoplasm, which may be neutrophilic, eosinophilic, or basophilic. Myelocytes are classified according to the granulation which they contain. A myelocyte with fine neutrophilic granules is designated as a neutrophilic myelocyte, because it will differentiate into the polymorphonuclear neutrophilic leucocyte. In the blood of the rabbit there are no neutrophiles and hence no neutro-

philic myelocytes in the bone marrow.

Two types of eosinophiles are recognized in the rabbit's blood; the true eosinophil and the pseudo-eosinophil. The latter is spoken of as the special cell and functionally corresponds to the neutrophil of man, (neutrophiles being phogocytic). The special cell is the cell that gives character to the blood of an animal. In the fully differentiated condition, both in the blood and bone marrow, the two types of eosinophiles are easily distinguished. The true eosinophil has large round granules, while those of the pseudo-eosinophil are smaller and typically rod shaped. Maximow thinks that in the rabbit both types of eosiniphiles originate from a common myelocyte, which may differentiate in one of two ways, either into an eosinophil or into a special cell.

Bone marrow smears of the rabbit prepared according to Pappenheim's method, the details of which have already been given, show great groups of eosinophilic and pseudo-eosinophilic myelocytes, some of which have both basophilic and eosinophilic granules in the same cell. Figures 4 and 5 from smears, stained in May Giemsa, are illustrative

of the one type of myelocyte with basophilic granules found in the bone marrow of the rabbit. The granules are seen to vary in size, but are generally rounded or slightly irregular and show no definite arrangement in the cytoplasm. This type of myelocyte is not to be confounded with the mast myelocyte, the precursor of the mast leucocyte, regardless of the avidity with which the granules take up basic stains. All of the granules in cells like those illustrated in figures 4 and 5 have a strong affinity for basic annilin dyes, in which respect they resemble the basophilic granules of mast cells. However, other cells whose general characters are similar to these (figs.4 and 5) contain a few granules which show a slight affinity for the acid component of the stain, which gives these granules a reddish violet color. Such a cell is shown in figure 6. In this instance the majority of the granules are still strongly basophilic, but among them are seen seven or eight granules which are reddish violet in color, a very different shade from the blue black color of the other granules. In figure 7 it is evident that the number

of reddish granules has increased; this is still more evident in figure 8, in which the reddish granules are very numerous. Figures 8 and 9 illustrate the general tendency of the reddish granules to accumulate in the widest part of the cell. In some cells the nucleus is sufficiently differentiated to be slightly polymorphous, or at least indented on one side. In these cells the reddish granules show a tendency to accumulate in the concavity of the nucleus. The reddish granules occupy this entire region, while the remaining portions of the cell are comparatively free from them (fig.8). However, figure 8 also shows that the reddish granules are not confined entirely to this region, as a few of them are seen scattered amongst the black granules in the other parts of the cell. Figures 8 to 10 show clearly that the granules spread outward from the region in which most of them are developed until they gradually fill the entire protoplasm. The figures show further, that as the reddish granules increase in number there is a corresponding decrease in the number of basophilic granules until the latter finally disappear

completely (figs.8 to 11).

At this point the question arises, are figures 4 and 5 typical mast myelocytes, in other words are the granules real basophilic granules? Figure 9 suggests that the number of basophilic granules is very gradually being reduced, but that there is a corresponding increase in the number of reddish violet granules, and that the primitive basophilic myelocyte is being differentiated into a granular cell, a type very different from what we naturally expect. Figures 10 and 11 are conclusive proof that the original myelocytes (figs.4 and 5) are not mast myelocytes, but give origin to leucocytes, very different from mast leucocytes, their granules eventually becoming acidophilic instead of remaining basophilic as they would if they were real mast myelocytes. The last figure of the series (fig.11) shows the fully differentiated eosinophil with the characteristic large round granules and polymorphous nucleus, which has been differentiated from a myelocyte with basophilic granules.

Benacchio used the same method and came to identical conclusions. However, he goes further and concludes that there are no other basophilic myelocytes in the bone marrow.

In the early basophilic myelocyte the granules, as previously stated, are not of the same size, some being large while others are small. The question naturally arises whether the basophilic granules, both large and small, are transformed into the eosinophilic or special granulation as the case may be? Or is it probable that the early basophilic granulation disappears gradually and is simultaneously replaced by a new granulation? From my observations I am fully convinced that there is such a gradual transformation of the early basophilic granules into the eosinophilic or special granule. Figures 5 to 7 show granules that are intermediate in staining reactions, having an affinity for both the acid and basic component of the staining mixture, which gives these granules a reddish violet color. Later the granules become more acidophilic, at the same time losing their affinity for the basic dye. The early basophilic granulation is in reality the true eosino-

philic or pseudo-eosinophilic granulation, although when first formed it consists of a predominantly basophilic element, so much so, that the granules do not stain in eosin. A gradual ripening process takes place, as Benacchio expresses it, during which process the strongly basophilic reaction of the granules is lost (figs.6 and 7).

In the earlier myelocyte stages (figs.4 and 5) in which all the granules are basophilic, there are very many small granules, with a few medium sized to large granules scattered among them. In the later myelocyte stages most of the basophilic granules are large, these larger ones in particular changing their staining reactions. However, the later stages, including those in which most of the granules are eosinophilic, contain a few small basophilic granules as well as some larger ones. The small granules in the earlier myelocyte stages are in all probability the youngest granules; all of them probably increase in size before developing an affinity for the acid dye. The presence of the small basophilic granules in the later myelocyte stages in which the acidophilic granules

are very numerous can probably be accounted for by assuming that new granules are still being formed, which at first are small and basophilic.

Benacchio apparently assumes this transformation of the basophilic granule into the eosinophilic, but he makes no specific statement in regard to it.

Pappenheim and St.Szecsi found similar cells containing two kinds of granules in the circulating blood of rabbits into which saponin had been injected. They concluded that cells in which some of the granules are basophilic are not mast leucocytes, but that they represent unripe eosinophilic and pseudo-eosinophilic leucocytes. The authors regard the basophilic granules of these cells as a "prodromale" or primitive granulation, which later disappears and is replaced by either the eosinophilic or special granulation. They conclude that in the rabbit the cells containing basophilic granules are not true mast cells. "Blutmastzellen sind also keine eigentliche zellart, sondern entweder abortivkörnig degenerierte Lymphoidzellen oder unreifkörnige sonstige Granulozyten." In

other words, the rabbit has no true mast leucocytes in its blood.

Benacchio used the smear method only. His method of study, in all probability accounts for the fact that he reports a total absence of both mast leucocytes and mast myelocytes in the bone marrow of the rabbit. Since I was unable to detect mast myelocytes and mast leucocytes by the smear method, paraffin sections were tried. These were stained in 50% alcoholic thionin, others in May Giemsa.

Kardos, in working with material fixed in Helly's mixture and 100% alcohol, found neither mast cells nor cells of any kind which contained basophilic granules. Paraffin sections were also studied in the present investigation, but with very different results from those obtained by Kardos. The technique for this has already been described. Contrary to his assumption, I found many cells with basophilic granules. In sections fixed in alcohol and stained in alcoholic thionin the basophilic granules are just as numerous as they are in the bone marrow smears (figs.12 and 13). When the alcoholic material was stained in May Giemsa the basophilic granules were not quite as numerous.

However, there were still many of these granules present in sections stained according to this method. After fixation in Helly's mixture no basophilic granules of any kind could be detected with any of the various stains used. This would indicate that the basophilic granules are soluble in water, but after alcoholic fixation many of the granules are able to resist the short exposure to water to which they are subjected while being stained in Giemsa. In the material fixed in Helly's mixture, the granules are exposed to the action of water for a long period of time which is sufficient to dissolve them.

Study of alcoholic material shows practically the same conditions as are seen in the bone marrow smears prepared according to Pappenheim's method. Many of the cells contain basophilic granules only, while others contain both basophilic and eosinophilic granules, and in still others all of the granules are eosinophilic. All possible intermediate stages between these different types of cells are as easily seen here as in the smears. Figures 12 and 13 from alcoholic thionin preparations of bone marrow illus-

trate one type of myelocyte with basophilic granules, which on closer investigation are found to be the same kind of cells as those in figures 4 and 5 from bone marrow smears, as all possible intermediate stages are observed.

May Giemsa preparations (figs.14 and 15) show the predominant basophilic granulation, but also a varying number of granules which show a slight reddish tinge (compare with bone marrow smears, figs.6 and 7). In figures 16 and 17, the reddish granules have increased in numbers, but with a corresponding decrease in the number of basophilic granules, until they finally disappear completely as is shown in figure 18 (compare with figs.9,10,11).

In addition to the numerous myelocytes with basophilic granules, which give origin to eosinophiles and special cells, (which Kardos failed to find), another type of basophilic myelocyte (which Kardos also failed to detect), must be considered (fig.19). In paraffin sections of bone marrow fixed in 100% alcohol and stained in alcoholic thionin mast leucocytes appear very different from unripe eosinophiles and pseudo-eosinophiles; furthermore it is possible to keep the two distinct even in the earlier

myelocyte stages. Eosinophilic and special myelocytes usually occur in groups, while the mast myelocytes are seen as cells which show no regular distribution. The nucleus of the mast myelocyte possesses a very thick nuclear membrane (fig.19), and contains a small amount of chromatin and a variable number of nucleoli or none at all. The basophilic granules have a remarkable affinity for basic annilin dyes in that they stain a bluish black in contradistinction to the reddish black of the eosinophil and special myelocyte granules (fig.12, a special myelocyte as compared with fig.19, a mast myelocyte).

I was fortunate to find a mast leucocyte and an eosinophil in the same field as shown in figure 20. The two cells are of approximately the same stage of differentiation, only that in the eosinophil (the upper figure) the entire nucleus is not seen. The basophilic granules of the eosinophil are very numerous and have a reddish black tinge, while the granules in the mastleucocyte are of an intense black and the nuclear membrane, even at this late stage, is comparatively thick. Figure 21 from an alcoholic thionin preparation, is illustrative of the

mast myelocyte with the characteristic basophilic granules. The nucleus is slightly polymorphous, possesses a thick nuclear membrane. In the fully differentiated mast leucocyte the nucleus is polymorphous, (contrary to Pröscher who claims that it is of the lymphocyte configuration) with a variable number of intensely colored basophilic granules in the cytoplasm (fig.22).

The Histogenous Mast Cells of the Rabbit.

Ehrlich, Westphal and Unna believe that histogenous mast cells are derived from fixed connective tissue cells. Ziegler says that they are polymorphous lymphocytes and elasmatocytes with metachromatic granules. Schreiber and Neumann claim that they are identical with clasmatocytes. Pappenheim and Downey find that histogenous mast cells are differentiated from lymphocytes in the adult, contrary to Maximow, who claims that they are differentiated very early in the embryo and the supply is maintained by mitosis of their own kind only. Downey and Weidenreich observed that in lymph glands of cat, small and medium sized lymphocytes differentiated mast granules. Downey found that lympho-

cytes with the characters of plasma cells gave origin to mast granules, material passing out from the nucleus in solution and forming the granules. The same author in a recent article has shown that in the guinea pig histogenous mast cells are derived from a type of cell similar to the clasmatocyte with a primitive or "prodromale" granulation. Whether the primitive granulation disappeared or became the final mast cell granulation is not known. Downey observed that in a developing mast cell, from a clasmatocytic type of cell, the metachromatic substance was formed within the nucleus and was confined entirely to the nucleus. Coincident with the formation of metachromatic substance within the nucleus the cytoplasmic granulation took on a decided reddish tinge, indicating that nuclear substance was passing out. "In the guinea pig there is no evidence of a nuclear degeneration, in the fully developed mast cell the nucleus is round or oval."

Sabrazes and Lafon have observed mitosis of mast cells in the horse, and Maximow in his investigations upon embryos and young cats and rats found mitosis of the same in a few instances. Mitosis may be regarded as one means

of increasing the numbers, but it is very probable that histogenous mast cells are differentiated from fibroblasts, lymphocytes, plasma cells, and clasmatocytes. Weidenreich and Downey have shown conclusively that undifferentiated elements may elaborate mast granules, contrary to Maximow, who believes that mast cells are differentiated very early in embryonic life and that all mast cells in the adult are descendants of the earlier ones.

Maximow found histogenous mast cells in all mammals investigated, even in the rabbit, "Wo sie von so vielen Autoren ganz geleugnet wurden." In the rabbit, "sind sie am schwächsten, bei Ratte and Maus dagegen am stärksten entwickelt." In regard to the significance of histogenous mast cells Maximow says, "Sicher musz also die Bedeutung der histiogenen Mastzellen für die Lebensfunktionen des Organismus grösser sein, als die der Mastleukocyten."

The general shape and size of mast cells may vary greatly. According to Weidenreich they are amoeboid and have phagocytic properties, as red blood corpuscles have been seen within them. Maximow finds that they are flattened out parallel to the surface and recommends cutting

sections for their study parallel to the surface (as previously stated). The nucleus is usually round or oval, separate masses of chromatin are often very difficult to detect.

In the abdominal wall of the rabbit histogenous mast cells are numerous in the upper layers of the cutis, less numerous in the lower layers, and are especially abundant around the hair follicles. In the intestine mast cells are also found; this type of cell, found only in the intestine, possesses a round nucleus and according to Maximow represents a special type of mast cell.

In turning to the origin of the histogenous mast cells in the adult rabbit we need not be concerned with the bone marrow. According to Maximow, "Bei allen untersuchten Säugetieren, ausser dem Kaninchen findet man im embryonalen Knochenmark sowohl 'histogene' also auch 'hamatogene' Mastzellen. Die ersten erscheinen früher, entstehen aus den lymphoiden Wanderzellen and sehen in jeder Beziehung den Mastzellen des Bindegewebes ähnlich; bei der Ratte bleiben sie im Mark auch beim erwachsen

Tier in grosser Anzahl, bei Katze und Meerschweinchen verschwinden sie später. Die Blutmastzellen enstehen im Mark ebenfalls aus den Lymphozyten durch allmählich granulaausarbeitung, aber später, und, wie es schient, als ganz besondere zellart, die zu den Bindegewebsmastzellen keine Beziegung offenbart, ausser der gemeinsamen ungranulierten Stammzelle."

From alcoholic thionin preparations of body wall of rabbit numerous mast cells in various stages of differentiation are recognized. The process of differentiation, from a cell of the clasmatocytic type, is identical with what Downey finds in the guinea pig. The cells of figures 23 and 24 are very similar to the ordinary type of clasmatocyte found in the loose connective tissues. Downey points out the fact, that the fully differentiated mast cell is very different from the clasmatocyte, although it is possible that the mast cell is differentiated from a clasmatocyte. Ranvier says that mast cells are a variety of clasmatocytes but clasmatocytes are not mast cells. In figure 23 numerous dark purple granules are seen distributed throughout

the cytoplasm of the cell. This type of granulation shows a barely perceptible affinity for basic annilin dyes. The granules are unusually uniform in size, and are developed in the cytoplasm of the cell. Downey found that in the lymph glands of cat, mast granules were differentiated in lymphocytes and plasma cells and that the nucleus was very actively concerned in the elaboration of the mast granules and that the fully differentiated mast cell possesses little chromatin. In the guinea pig he also found that the nucleus participates in the formation of mast cells. In figure 24 metachromatic substance is being formed within the nucleus and is limited to the nucleus. A few metachromatic particles are seen on the outer edge of the nuclear membrane, but the primitive granulation remains unchanged. Maximow reports having seen the same thing in the rabbit and the guinea pig and that with alcoholic thionin the karyoplasm of the nucleus takes on a metachromatic violet tinge. Within the nucleus there are large metachromatic particles, and the same particles may be found hanging on to the nuclear membrane. Maximow favors the idea that mast cells are formed in em-

bryonic life only, and that the supply is maintained exclusively by mitosis of their own kind. He recognizes the presence of metachromatic substance in the nucleus, but does not interpret that fact in the sense of progressive differentiation of mast granules in non-granular cells.

The origin of the primitive grabulation is not very clear as it is already present when the nucleus contains only a small amount of metachromatic substance (fig.24). However, as more metachromatic substance is formed within the nucleus and passes out into the cytoplasm the original or primitive granulation immediately becomes metachromatic also (fig.25). Downey favors the view that the primitive grabulation consists of a combination of the metachromatic substance from the nucleus with the primitive granules.

Summary:-

Weidenreich has demonstrated that in man the mast leucocyte is a degenerate lymphocyte, the mast granules in this instance are derived from nuclear buds which are extruded into the cytoplasm.

Benacchio comes to no definite conclusion in regard to the origin of mast leucocytes in the rabbit. He believes that the myelocytes with basophilic granules in the bone marrow of rabbit are not true mast myelocytes, but are unripe eosinophiles and pseudo-eosinophiles in which the early granulation is basophilic. He concludes: "Unentschieden ist noch die Frage über die Entstehung der Mastzellen des Blutes. Ihre Abstammung ist unbekannt. Die ersten Zellen (in this instance the Basophilic myelocytes) sehen 'prima vista' aus wie Mastmyelozyten mit basophilen metachromatischen granulationen, erst die weitere Beobachtung zeigt, dasz sie Vorstufen der eosinophilen Zellen resp. Granulationen sind." Further, "dasz das Dogma, das Knochenmark echte Mastleukozyten führt, falsch ist. Zellen mit basophil-metachromatischen Granulationen, wie Mastzellen solche führen müszten, sind nicht vorhanden."

Contrary to Kardos, who fails to find myelocytes of any description with basophilic granules, it is possible to demonstrate in alcoholic thionin preparations two types of myelocytes in the bone marrow of the rabbit. Each myelocyte type has its own peculiar and distinct line of differentiation.

The eosinophil and pseudo-eosinophil is represented in the bone marrow by a myelocyte with a varying number of basophilic granules which in the early formed condition have a remarkable affinity for basic annilin dyes. The basophilic granulation in this type of myelocyte is not permanent, a gradual ripening process takes place during which it is transformed into the eosinophilic or pseudo-eosinophilic granulation. Therefore, the precursor of the eosinophil and special cell of the rabbit should not be designated as a mast myelocyte, but as a myelocyte which contains unripe granules which are basophilic only during their early stage of differentiation.

Contrary to the statements of Benacchio and Kardos, it is possible to demonstrate a second type of myelocyte in the bone marrow of the rabbit, which is the mast myelocyte, the precursor of the mast leucocyte. The granules undergo no transformation such as was described for the first type of myelocyte. In alcoholic thionin preparations mast myelocytes and mast leucocytes are easily distinguished from the other myelocytes and fully differentiated eosinophiles and special cells.

The mast leucocyte of the rabbit is not a degenerate type of cell formed within the circulation as Pröscher states, but is a true granular cell with it's own myelocyte stage in the bone marrow.

Histogenous mast cells are not derived exclusively from pre-existing mast cells, but are also differentiated from a clasmatocytic type of cell with a primitive granulation. Within the nucleus a metachromatic substance is formed from the chromatin which is extruded into the cytoplasm. Coincident with the extrusion of metachromatic substance the primitive granules take on a reddish violet tinge. It could not be decided whether the material discharged from the nucleus is the permanent granulation, or whether the primitive granules combine with the metachromatic substance and remain as the final granulation.

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